

## Chelex DNA Preparation Protocol for "Modern Tissue"

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(\*As modified for cetacean tissues by F. Cipriano...)

Based on the protocol of: Walsh, S. P., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10(4): 506-513. See also Morin, P. A. and D. S. Woodruff. 1992. Paternity exclusion using multiple hypervariable microsatellite loci amplified from nuclear DNA of hair cells. Pages 63-81 in R. D. Martin, A. F. Dixon, and E. J. Wickings (eds). *Paternity in primates: genetic tests and theories*. Basel, Karger. Chelex 100 is available from Biorad (1-800-4BIORAD, (BioRad Chelex 100 Resin, Biotechnology Grade, 100-200 mesh, sodium form, Catalog 143-2832 for 100 g).

1) Obtain a CLEAN \*10% Chelex tubes from the fridge (protocol below), and turn on the heating block (or PCR machine). Fill the wells in the heating block with water so block is never more than 100 C. Don't fill the wells in the PCR machine!

2) Aliquot \*80-100 ul of the 10% Chelex solution into clean 600 ul tubes (or whatever fits your heating block or PCR machine) - one tube for each sample to be extracted (\*Shake the 10% tube to resuspend Chelex before each pipet draw, since the Chelex tends to settle quickly.)

3) Place a TINY amount of tissue into the Chelex tube(s). \*A speck of dolphin skin or meat about 1 mm across is enough! \*2 ul of preserved or fresh cetacean blood will also work (cetacean blood can be preserved in a 50:50 mix with salt-saturated DMSO). \*To expose more surface area of the tissue to Chelex, scrape the tissue across a new piece of sterile weighing paper with a sterile scalpel blade, flattening it out several times till it forms a little flake. Place the flake into the tube with Chelex. Flame-sterilize the scalpel blade and any forceps used (dip in ethanol, flame in alcohol lamp, repeat) and replace weighing paper between samples to prevent cross-contamination.

4) Incubate in the heating block (or PCR machine) at 100 C for \*20 minutes. Shake up tubes after 10 minutes if you remember.

6) While waiting, you can prepare your PCR master mix (some people call it the "cocktail").

7) After about 20 minutes, take your Chelex tubes off the heating block or out of the PCR machine.

8) Spin down the Chelex tubes briefly so that condensate, Chelex, and tissue speck are forced to bottom of tubes, leaving hopefully-clear supernatant above (pigmented samples may still work). Use 1-2 ul of Chelex supernatant (AVOID THE BEADS) as amplification template in each PCR sample tube. Thermocycle with normal parameters.

9) Store Chelex extractions in the FREEZER for use in other PCR reactions. REFRIGERATED CHELEX SAMPLES DO NOT LAST! It may even be more effective to pipet off the supernatant to a new tube, free of Chelex, and store THAT frozen. If your Chelex extraction doesn't work, add another 80-100 ul of Chelex and boil again for 20 minutes. If that doesn't work, start over with a new bit of tissue.

### Preparation of 10% Chelex 100 Aliquots

1) Obtain a new 50 mL polyethylene (clean, sterile, Falcon - type) conical tube.

2) Fill the conical tube to the 40 mL mark with dH<sub>2</sub>O.

3) Weigh out (sterile weighing paper, tap out Chelex, NO SPATULA IN BOTTLE) 4 grams of Chelex 100. Pour into Falcon tube.

4) Using a P1000 pipet (aerosol resistant or "filtered" pipette tips if possible), aliquot the 10% Chelex into 1.7 mL micro-centrifuge tubes obtained from a freshly opened bag. You will need to cap and shake the tube constantly to keep Chelex suspended.

Put about 1.0 ml into each tube so that tubes are used for only one extraction set (about 10 samples). Compare the level of Chelex in all the tiny little tubes to be sure that they're all made up to about 10% (exact proportion doesn't matter).

5) Store prepared tubes in the refrigerator.